



## Effects of Different Lipid Sources in Total Parenteral Nutrition on Whole Body Protein Kinetics and Tumor Growth

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**ABSTRACT.** This study examined the short-term effects of three total parenteral nutrition solutions, each containing a different lipid source, on host and tumor protein metabolism in a rat cancer model. Each diet contained 220 kcal/kg per day, including 2 g of nitrogen/kg per day and 50% of nonprotein calories as either a structured lipid of medium-chain triglycerides and fish oil, a physical mix of medium-chain triglycerides and fish oil, or Liposyn II, a long-chain triglyceride. A 3-day intravenous feeding infusion began on day 7 after tumor implantation. Tumor growth rate, nitrogen balance, energy expenditure, and plasma albumin, glucose, and free fatty acids were measured, and whole body protein kinetics and fractional synthetic rates in liver, muscle, and tumor tissues were assessed using a constant infusion of  $^{14}\text{C}$ -leucine. The results revealed that tumor growth rate was slowed in structured lipid-fed

animals ( $p = .06$ , one-way analysis of variance) with significant increases in rates of tumor protein synthesis and tumor protein breakdown ( $p < .001$ , one-way analysis of variance). Although muscle fractional synthetic rates were significantly decreased in tumor-bearing animals ( $p < .05$ , two-way analysis of variance), the rates in structured lipid-fed animals were restored. Nitrogen balance improved significantly in structured lipid-fed animals. The results demonstrate that the source of lipid in total parenteral nutrition solutions can influence tumor and host protein metabolism, and that a structured lipid composed of medium-chain triglycerides and fish oil seems to improve protein metabolism in host tissue without stimulating tumor growth. (*Journal of Parenteral and Enteral Nutrition* 16:545-551, 1992)

Malnutrition and weight loss leading to cancer cachexia are predictive of a poor prognosis.<sup>1,2</sup> Total parenteral nutrition (TPN) regimens have been employed to reverse or at least prevent further weight loss in these patients. However, results of studies exploring the role of TPN in cancer have been inconsistent and/or inconclusive.<sup>3</sup> Manipulating the source of calories in TPN by substituting some long-chain triglycerides (LCTs) for glucose has not improved repletion of lean tissue and similarly leads to deposition of fat in patients with cancer cachexia.<sup>2,3</sup> LCTs in large amounts also have the potential for detrimental effects, including impairment of the reticulo-endothelial system,<sup>4</sup> diminished bacterial clearance,<sup>5</sup> and fatty infiltration of the liver. Moreover, TPN as currently formulated may favor tumor growth.<sup>6,7</sup>

Medium-chain triglycerides (MCTs), composed of fatty acids essentially of chain lengths 8 to 10 carbon atoms, are available as a fat source in TPN with some experimental evidence for their potential value in cancer cachexia.<sup>8</sup> MCTs are rapidly available to tissues and preferentially oxidized.<sup>9</sup> This lipid thus provides an efficient fuel source with little or no deposition as body fat.<sup>10</sup> However, MCT-enriched emulsions may clear too rapidly, do lack essential fatty acids, and are less well tolerated when used as a sole lipid source. To help rectify

this problem a unique method has been developed to reesterify both long-chain and medium-chain fatty acids on the same glycerol backbone by an initial hydrolysis of a mixture of medium- and long-chain triglycerides and random reesterification to produce a new form of lipid, a structured lipid. Structured lipids can retain most of the desired qualities of both types of fatty acids while reducing the adverse effects of each.<sup>11</sup>

Studies in our laboratory using a structured lipid composed of MCTs and LCTs compared with LCTs alone in parenterally fed thermal injury models have shown an attenuation of protein catabolism and improved serum albumin concentrations.<sup>12</sup> In a rat cancer model a similar structured lipid improved nitrogen balance and increased muscle protein synthetic rates compared with LCTs.<sup>13</sup> In a recent study, a structured lipid composed of MCTs and fish oil reduced net protein catabolism, decreased total energy expenditure, and improved cumulative nitrogen balance compared with safflower oil in enterally fed burned rats,<sup>14</sup> suggesting that the use of fish oil as the source of LCTs in a structured lipid may confer additional beneficial effects. It is well established that the key components of fish oil, eicosapentaenoic acid and docosahexaenoic acid, compete with arachidonic acid metabolism for the cyclooxygenase pathway of eicosanoid metabolism,<sup>15,16</sup> which can influence the immune function of the host. Feeding studies also have demonstrated that fish oil causes later tumor appearance rates and lowers tumor incidence.<sup>17</sup>

Based on these findings, we examined the short-term

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effects of TPN feeding with a structured lipid composed of MCTs and fish oil (60/40, w/w) on tumor growth and protein metabolism in a rat Yoshida sarcoma model. LCT (safflower oil) emulsion was used as a control diet. In addition, we also studied the effects of a physical mix of MCTs and fish oil (50/50, w/w) to assess the independent effects of structuring a lipid.

### METHODS

Forty-nine male Sprague-Dawley rats weighing 65 to 100 g (Taconic Farms, Germantown, NY) were housed in suspension cages, two per cage, in a light- and temperature-controlled room (12 hour light/dark cycle, 24 to 26°C). In the later part of the study, animal body weights at arrival time were approximately 20 g above the desired range. In addition, our shipment of structured lipid (SL) emulsion was delayed and not delivered until after the study had already begun. As a consequence, more animals in the SL groups tended to have higher initial body weights when enrolled in the study. All rats were allowed to consume standard laboratory chow (Charles River D-3000, Agway Agricultural Products, Minneapolis, MN) and tap water *ad libitum* 5 days before the start of the experiment. On day 0 the animals were weighed and received subcutaneous injections into the right flank of either  $10^7$  cells of Yoshida sarcoma ( $n = 25$ ) or an equal volume of sterile saline ( $n = 24$ ). On day 7 all animals underwent internal jugular vein catheterization under ether anesthesia using Silastic catheters (.025 inner diameter  $\times$  .047 outer diameters, Dow Corning Laboratories, Midland, MI). The catheter was attached to a flow-through swivel (Instech Laboratories, Philadelphia, PA) that allowed for free movement of the rat and constant infusion. After surgery the rats were housed singly in individual wire suspension cages, infused with 0.9% sterile saline for 2 to 3 hours, and allowed to recover.

Animals were randomly assigned to one of three diets, which were identical except for their lipid source. The diets contained as a lipid source either LCTs (LCT group) (LCTs were given as Liposyn II, Abbott Laboratories, North Chicago, IL), an emulsion of a physical mix of MCTs and menhaden fish oil (FO) (50/50 w/w, PM group), or an emulsion of structurally rearranged lipid molecules of MCTs and FO (60/40 w/w, SL group). (Oils for SL and PM were provided by Capital City Products, Inc., Columbus, OH. Emulsions were prepared by CER-NEP Synthelabo, Cedex, France.) Diets were mixed in the hospital pharmacy and were isocaloric, isonitrogenous, and isovolumetric, providing 220 kcal/kg per day, with 2 g of nitrogen/kg per day (Table I). Fifty percent of nonprotein calories were given as fat, with the balance as dextrose. After recovering from surgery the animals were infused with their respective diets at a flow rate of one-half the planned rate to allow for adaptation to TPN. On the morning of day 8 infusion rates were increased to the full rate. All animals were weighed at the beginning and the end of the 3-day infusion to determine any changes in body weight. Twenty-four-hour urine samples were collected for nitrogen balance determination.

For the last 4 hours of parenteral feeding on day 10 the rats were transferred to enclosed metabolic chambers

TABLE I  
Dietary intake

	Structured lipid*	Physical mixture†	Long-chain triglycerides
Total calories	220	220	220
Amino acids‡	50	50	50
Dextrose	85	85	85
Lipids	85	85	85

Values are kcal/kg of body weight.

Additives/1000 mL were sodium chloride, 30 mEq; sodium acetate, 30 mEq; potassium chloride, 30 mEq; potassium acetate, 25 mEq; potassium phosphate, 16 mEq; calcium gluconate, 8.4 mEq; magnesium sulfate, 8.0 mEq; trace mineral mix (Ascot Pharmaceuticals, Inc., Skokie, IL), 10.2 mL. 0.50 mL each of MVC 9 + 3 multivitamins (Lyphomed, Inc., Melrose Park, IL) and choline chloride (w/v) were added per 100 mL of hyperal solution.

\* Structured lipid: medium-chain triglycerides and menhaden fish oil 60/40, w/w.

† Physical mix: medium-chain triglycerides and menhaden fish oil, 50, 50, w/w.

‡ Crystalline amino acids (Travasol, Baxter-Travenol Laboratories, Deerfield, IL).

to measure energy expenditure and leucine kinetics. A tracer amount of L- $^{14}$ C]leucine (1.5  $\mu$ Ci/h) (New England Nuclear, Boston, MA) was added to the diet solutions, which were then infused at 1.25 mL/h using a Harvard pump (Harvard Apparatus, South Natick, MA). During the tracer infusion, carbon dioxide production, oxygen consumption, and total energy expenditure were measured as described previously.<sup>18</sup>

At the end of the infusion, rats were killed by decapitation and arterial/venous blood was collected in heparinized tubes; plasma was separated by centrifugation. The whole liver was removed and weighed. A portion of the abdominal rectus muscle was removed. Two approximately 1-g pieces of each type of tissue were individually frozen. The tumor was weighed and frozen intact. All samples were stored at -25°C until analysis.

### Analytical Methods

Plasma albumin concentrations were measured by the bromocresol green method (Albustrate, General Diagnostics, Morris Plains, NJ).

Plasma leucine specific activity was measured as previously described.<sup>18</sup> Plasma leucine concentrations were determined by high-performance liquid chromatography. Radioactivity in the form of  $\alpha$ -ketoisocaproic acid was removed by treatment with  $H_2O_2$ .<sup>19</sup> Tissue samples were homogenized and separated into an acid-soluble fraction and a precipitate by treatment with sulfosalicylic acid. The specific activity of free leucine in the acid-soluble fraction of muscle, liver, and tumor tissue homogenate (Si) was determined by a procedure similar to that for plasma. An external standard was used for efficiency determinations, and the random coincidence monitor was considered and corrected. The acid-insoluble fractions of these tissues were dried and solubilized in a 0.1 N quaternary ammonium hydroxide solution (Beckman BTS-450, Beckman Instruments) and counted for  $^{14}$ C leucine contents, using internal standards for efficiency determinations.

Total energy expenditure was measured using the equation of de Weir.<sup>20</sup> Tissue nitrogen and total urinary

nitrogen were determined using the micro-Kjeldahl method.<sup>15</sup>

#### Calculations

Rates of plasma leucine flux were determined from the equation<sup>21</sup>

$$Q = I/Sp$$

where Q (flux) is the rate of appearance of leucine in the plasma compartment expressed in micromoles per hour, I is the rate of infusion of isotope in disintegrations per minute per hour and Sp is the specific activity of leucine at steady state in disintegrations per minute per micromole.

Whole body leucine kinetics were calculated using the equation

$$Q = I + B = O + S$$

where Q is the same as above and I represents dietary intake; S, the incorporation of leucine in protein synthesis; and B, the rate of leucine appearance due to protein breakdown, were calculated. The rate of plasma leucine oxidation, O, was estimated from the appearance of <sup>14</sup>CO<sub>2</sub> in expired breath from the equation

$$O = E/(K \cdot Sp)$$

where O is total plasma leucine oxidized in micromoles per hour, Sp is the specific activity of leucine at steady state, E is the rate of <sup>14</sup>CO<sub>2</sub> expired in the breath in disintegrations per minute and K is a constant to correct for <sup>14</sup>CO<sub>2</sub> produced in tissues not appearing in breath.

Fractional synthetic rates of protein in liver, muscle, and tumor were calculated from the ratio of leucine specific activity in the protein bound fraction to that in the intracellular free fraction using Garlick's equation<sup>21</sup>

$$\frac{S_b}{S_i} = \frac{\lambda_i}{(\lambda_i - K_s)} \cdot \frac{(1 - e^{-K_s t})}{(1 - e^{-\lambda_i t})} - \frac{K_s}{(\lambda_i - K_s)}$$

where K<sub>s</sub> is the fractional rate of protein synthesis (FSR), λ<sub>i</sub> is the rate of increase of tracer content in the acid soluble fraction, and t is the duration of tracer infusion.

The rate of leucine utilization in tissue protein synthesis (Ts) was calculated according to the equation:

$$Ts = FSR \cdot L_m$$

where FSR is calculated as above and L<sub>m</sub> is the mass of leucine in the tissue under consideration.<sup>22</sup>

The rate of tissue protein breakdown (Tb) is calculated from the following equation<sup>22</sup>:

$$Tb = (q \cdot I(Sp - Si))/(Si \cdot Sp)$$

where q is the fraction of infused tracer distributed to the tissue, I is the rate of constant infusion of tracer in disintegrations per minute per hour, and Sp and Si are the specific activities of plasma and free intracellular leucine, respectively, at steady state.

Estimates of fractional tumor growth were derived from tumor volume measurements on day 7 and 10. Tumor volumes were estimated from measurements of

tumor length, width, and depth in millimeters. These measurements bear a close relationship to tumor weight.

#### Statistical Analysis

Results are presented as mean ± SEM. Statistical analysis of the effect of diet and tumor on protein metabolism was done using two-way analysis of variance (ANOVA) utilizing a statistical software program (BMDP Statistical Software, Los Angeles, CA). The effect of diet on tumor growth and tumor protein kinetics measurements was compared using a one-way ANOVA.

#### RESULTS

In the present study, the calories provided were less than would be consumed by *ad libitum* feeding for a similar size, non-tumor-bearing rat, but this is dictated by animal tolerance for parenteral feeding. In addition, the stress of surgery may also cause a decrease in weight during the first few days after catheterization. Therefore, after 3 days of intravenous feeding all animals lost weight regardless of their feeding solutions (data not shown). Because the initial body weights were higher in both SL feeding groups despite random allocation, the effect of diet on weight change was expressed as the percent of weight changed (weight change/initial weight). It was found that there were no significant differences among all diets in the percent of weight changed (data not shown).

Table II shows daily and cumulative nitrogen balance for all six groups during parenteral feeding. On the eighth day after implantation, all groups had a negative nitrogen balance, which is presumed to be because of the effect of surgical stress and the initially inadequate caloric intake during the period of TPN adaptation. However, nitrogen balance returned to positive levels by the second day of feeding. Nitrogen balance for the final feeding day (day 10) and cumulative nitrogen balance (days 8, 9, and 10) were significantly different by diet (*p* < .05, two-way ANOVA), where SL-fed animals had the highest balances.

Neither the tumor percent protein content, total tumor weight, nor tumor weight as a percent of body weight differed among the groups (Table III). Tumor fractional protein synthetic rate (FSR) and Si/Sp ratio of SL animals were significantly higher than both PM and LCT groups (*p* < .001, one-way ANOVA). Compared with LCT animals, the SL group had a higher rate of tumor protein synthesis (Ts) (*p* < .005, one-way ANOVA) and an increased tumor protein breakdown rate (Tb) (*p* < .001, one-way ANOVA). A higher tumor Ts would usually indicate increased tumor growth. In this case, however, the high rate of tumor protein synthesis is offset by a high rate of tumor protein breakdown, so that the net protein accumulation of tumor tissue (Ts - Tb) in the SL group was not significantly different from the other groups. The measured tumor growth rate in the SL group was marginally different (*p* = .06, one-way ANOVA) from the other groups. However, by Student's *t* test tumor growth rate was lower in SL vs PM and LCT (both *p* < .05).

TABLE II  
Daily and cumulative nitrogen balance  
(mg nitrogen)

	Day 8	Day 9	Day 10*	Day 8, 9, 10*
T SL	-22.2 ± 4.7 (10)	54.7 ± 6.2 (10)	77.4 ± 9.9 (9)	35.2 ± 8.0 (29)
NT SL	-5.1 ± 7.7 (9)	62.9 ± 16.1 (10)	124.7 ± 14.7 (10)	63.1 ± 12.5 (29)
T PM	-6.5 ± 8.8 (9)	42.6 ± 19.0 (10)	60.5 ± 9.2 (9)	32.5 ± 9.3 (28)
NT PM	-37.6 ± 7.2 (9)	35.0 ± 12.1 (9)	53.6 ± 14.2 (9)	17.0 ± 10.1 (27)
T LCT	-16.7 ± 7.5 (8)	51.9 ± 14.1 (8)	79.3 ± 11.5 (8)	38.1 ± 10.5 (24)
NT LCT	-5.7 ± 14.4 (7)	23.8 ± 12.1 (7)	25.4 ± 21.5 (7)	14.5 ± 9.6 (21)

Values are milligrams of nitrogen, mean ± SEM.

T = tumor-bearing; NT = non-tumor-bearing; SL = structured lipid diet; PM = physical mix diet; LCT = long-chain triglyceride diet. Numbers in parenthesis represent n.

\*  $p < .05$  by diet (two-way ANOVA).

TABLE III  
Tumor leucine kinetics, tumor growth rates, and tumor weights

	SL	PM	LCT
Total protein	0.74 ± 0.0 (9)	0.79 ± 0.11 (8)	0.85 ± 0.10 (8)
Protein (%)	15.5 ± 0.5 (9)	15.8 ± 1.0 (8)	15.6 ± 0.1 (8)
FSR (%/day)*	26.2 ± 1.8 (8)†	10.4 ± 1.2 (8)	8.3 ± 1.7 (8)
Growth rate (%/day)‡	20.7 ± 3.2 (8)†	30.5 ± 3.6 (8)	31.1 ± 3.1 (8)
Ts (μmol/h·g)§	0.85 ± 0.06 (9)	0.71 ± 0.12 (8)	0.43 ± 0.03 (7)†
Tb (μmol/h·g)§	0.28 ± 0.04 (9)	0.23 ± 0.05 (8)	0.06 ± 0.03 (8)
Ts·Tb (μmol/h·g)	0.57 ± 0.07 (8)	0.48 ± 0.08 (8)	0.40 ± 0.04 (7)†
Si/Sp*	0.38 ± 0.0 (9)	0.52 ± 0.05 (8)	0.82 ± 0.06 (8)
Weight (g)	4.8 ± 0.6 (9)	5.2 ± 0.7 (8)	5.6 ± 0.7 (8)
% Body weight	3.8 ± 0.5 (9)	4.4 ± 0.6 (8)	4.7 ± 0.5 (8)

Values are mean ± SEM; SL = structured lipid diet; PM = physical mix diet; LCT = long-chain triglycerides; FSR = protein fractional synthetic rate; Ts = rate of protein synthesis; Tb = rate of protein breakdown; Ts·Tb = net protein accumulation; Si/Sp = ratio of leucine specific activity in acid soluble tissue fraction to plasma specific activity at isotopic steady state.

Numbers in parenthesis represent n.

\*  $p < .001$  by diet (one-way analysis of variance (ANOVA)).

† Statistical outliers were removed according to the Q test.

‡  $p = .06$  by diet (one-way ANOVA).

§  $p < .01$  by diet (one-way ANOVA).

TABLE IV  
Whole body leucine kinetics

	n	Plasma leucine* (μmol/mL) (n)	Flux (μmol/h·100 g)	Synthesis* (μmol/h·100 g)	Breakdown (μmol/h·100 g)	Oxidation (μmol/h·100 g) (n)	% Flux*† oxidized	Isotope balance† (μmol/h·100 g) (n)	Leucine clearance* (mL/h) (n)
T SL	9	0.13 ± 0.01 (8)‡	48.7 ± 5.7	31.2 ± 3.2	22.7 ± 5.3	17.5 ± 2.8 (8)‡	27.6 ± 2.0	10.5 ± 1.8 (7)‡	393.8 ± 35.8 (8)‡
NT SL	10	0.13 ± 0.01 (9)‡	49.9 ± 3.0	36.1 ± 2.3	23.9 ± 3.0	13.9 ± 1.1	20.3 ± 1.3	12.3 ± 1.1	393.1 ± 40.2
T PM	8	0.19 ± 0.04	46.8 ± 2.6	32.3 ± 2.0	16.0 ± 3.0	14.4 ± 1.0	26.1 ± 1.7	16.3 ± 1.6	302.9 ± 51.9
NT PM	8	0.12 ± 0.01 (7)‡	47.2 ± 3.5	32.2 ± 3.2	16.6 ± 2.9	15.0 ± 1.3	26.7 ± 1.7	15.6 ± 1.3	363.1 ± 29.9 (7)‡
T LCT	8	0.17 ± 0.01	41.5 ± 2.8	25.8 ± 1.2	11.7 ± 2.2	15.8 ± 2.0	32.0 ± 2.7	14.1 ± 1.4	233.0 ± 28.3
NT LCT	6	0.14 ± 0.01	54.5 ± 6.6	36.4 ± 5.1	21.8 ± 4.5	18.0 ± 2.0	27.0 ± 1.3	14.7 ± 2.6	405.7 ± 43.5

Values are mean ± SEM. Abbreviations are as in Table III.

\*  $p < .05$  T vs NT (two-way ANOVA).

†  $p < .05$  by diet (two-way ANOVA).

‡ Statistical outliers were removed according to the Q test.

Whole body leucine kinetics are shown in Table IV. There were no differences among the groups in either leucine flux, the rates of leucine oxidation, or leucine breakdown. However, tumor-bearing rats had a lower rate of synthesis and a significantly higher percent of plasma flux oxidized than did non-tumor-bearing rats, indicating a catabolic metabolism in tumor-bearing animals. Furthermore, a higher plasma leucine concentration was found in tumor-bearing animals, reflecting the significant decrease in leucine clearance in these animals. A significant reduction in the percent of flux oxidized was also seen in non-tumor-bearing animals, as well as in animals receiving SL. Isotope balance measurements were significantly affected by diet ( $p < .05$ , two-way ANOVA), with the SL group having the lowest value.

Table V lists liver protein contents, weights, and estimates of leucine kinetics. A significant difference due to diet administration was seen in total liver weights ( $p < .001$ , two-way ANOVA) and liver weight as a percent of body weight ( $p < .05$  by diet, two-way ANOVA), where highest values were found in those groups receiving fish oil, either as SL or PM. In addition, liver weights as a percent of body weight were significantly higher in tumor-bearing animals ( $p < .05$ , two-way ANOVA) than in non-tumor-bearing ones. The increase in liver size of SL animals is attributed to their greater total protein mass ( $p < .001$  by diet, two-way ANOVA). The significant changes in the rate of protein synthesis (Ts) ( $p < .001$  by diet, two-way ANOVA) were reflected in the FSRs ( $p < .001$  by diet, two-way ANOVA). No changes

TABLE V  
Protein content and estimates of leucine kinetics in liver

	n	Liver* weight (g)	Liver weight† (% body wt)	Total protein* (g)	FSR‡ (%/day) (n)	Si/Sp*	Ts* (μmol/h·g) (n)	Tb (μmol/h·g) (n)	Ts-Tb (μmol/h·g) (n)
T SL	9	6.4 ± 0.2	5.0 ± 0.2	1.30 ± 0.04	40.6 ± 5.3	0.48 ± 0.05	2.48 ± 0.33	2.27 ± 0.40	0.20 ± 0.24
NT SL	10	5.8 ± 0.1	4.3 ± 0.1	1.22 ± 0.02	41.8 ± 2.8 (9)	0.50 ± 0.07	2.62 ± 0.16 (9)§	2.14 ± 0.35 (9)§	0.48 ± 0.29 (9)§
T PM	8	5.8 ± 0.1	4.9 ± 0.2	1.11 ± 0.05	25.0 ± 4.1	0.72 ± 0.10	1.45 ± 0.24	1.24 ± 0.50	0.21 ± 0.32
NT PM	8	5.9 ± 0.3	4.9 ± 0.2	1.19 ± 0.04	25.9 ± 6.3	0.65 ± 0.09	1.53 ± 0.34	1.78 ± 0.76	-0.25 ± 0.45
T LCT	8	5.2 ± 0.2	4.4 ± 0.2	1.03 ± 0.04	13.4 ± 2.1	0.93 ± 0.12	0.80 ± 0.12	0.37 ± 0.32	0.43 ± 0.26
NT LCT	6	5.4 ± 0.2	4.3 ± 0.1	1.11 ± 0.03	29.1 ± 4.2	0.52 ± 0.04	1.80 ± 0.24	2.34 ± 0.31	-0.55 ± 0.19

Values are mean ± SEM. Abbreviations are as in Table III.

\*  $p < .001$  by diet (two-way analysis of variance [ANOVA]).

†  $p < .05$  by diet and T vs NT (two-way ANOVA).

‡  $p < .05$  by diet (two-way ANOVA).

§ Statistical outliers were removed according to the Q test.

were seen in either liver protein breakdown rate (Tb) or net protein gain (Ts-Tb). Si/Sp ratio was highest in tumor-bearing animals ( $p < .05$ , tumor-bearing vs non-tumor-bearing, two-way ANOVA) and in animals infused with the LCT diet ( $p < .05$  by diet, two-way ANOVA).

In the muscle tissue (Table VI), although no changes occurred in any of the groups' rates of protein synthesis (Ts), protein breakdown (Tb), or the ratio of Si/Sp, tumor-bearing groups had a decrease in net protein accumulation (Ts-Tb) ( $p < .05$  T vs NT, two-way ANOVA). The FSR was also markedly lower in tumor-bearing animals than in non-tumor-bearing animals ( $p < .05$  T vs NT, two-way ANOVA), indicating the catabolic state of those animals having tumors. In contrast, however, animals administered SL had a higher muscle FSRs compared with the other tumor-bearing groups. Thus, SL seems to prevent the tumor-induced reduction in muscle protein synthesis. Percent protein content was higher in animals receiving the physical mix diet ( $p < .001$  by diet, two-way ANOVA).

Respiratory quotient (RQ), energy expenditure, plasma albumin, plasma glucose, and free fatty acid concentrations are shown in Table VII. RQ and energy expenditure were not affected by either diet administration or the presence of tumor. Structured-lipid-fed animals had the lowest plasma albumin levels ( $p < .001$  by diet, two-way ANOVA) and the highest free fatty acid levels ( $p < .01$ , two-way ANOVA). Tumor-bearing animals maintained significantly higher concentrations of free fatty acids ( $p < .01$ , two-way ANOVA) and glucose ( $p < .05$ , two-way ANOVA) compared with non-tumor-bearing animals.

## DISCUSSION

The present study demonstrates that different lipid sources in TPN solutions can influence tumor and host metabolism, as well as tumor growth. After 3 days of intravenous feeding using either conventional LCTs, a physical mixture of MCTs and fish oil (PM) or MCT-fish oil SL, more positive daily and cumulative nitrogen balances ( $p < .05$ , two-way ANOVA) were observed in animals receiving SL-enriched TPN (Table II). In the non-tumor-bearing animals, this achievement was associated with a significant decrease in percent of plasma flux oxidized (Table IV), increased rates of liver FSR and Ts, and a larger liver (Table V). For the tumor-bearing animals, SL feeding resulted in a larger liver, a higher liver FSR, and marginal reduction in tumor growth. Although SL feeding induced a higher protein turnover in the tumor tissue, the tumor net protein accumulation in these animals was not different compared with the PM- and LCT-fed groups. These results indicate that the effects of SL on whole body nitrogen utilization in tumor-bearing animals importantly reflects that more nitrogen was retained in the host but not at a cost of enhanced tumor growth.

The results of this study agree with a previous report<sup>23</sup> from our laboratory, confirming that the lipid source in TPN can influence tumor and host protein kinetics after a short feeding period. There were differences between the two studies, however. In the previous study, SL did not affect tumor protein breakdown, but instead significantly decreased tumor protein synthesis.<sup>23</sup> In addition, the increase in muscle protein synthetic rates seen in this study was not observed previously. Although the

TABLE VI  
Protein content and estimates of leucine kinetics in muscle

	n	% Protein*	FSR‡ (%/day) (n)	Si/Sp	Ts (μmol/h·g)	Tb (μmol/h·g)	Ts-Tb† (μmol/h·g)
T SL	9	19.5 ± 0.6	4.9 ± 0.9 (8)	0.39 ± 0.08	0.26 ± 0.05	0.27 ± 0.05	-0.018 ± 0.02
NT SL	10	19.1 ± 1.8	4.2 ± 0.6 (9)†	0.58 ± 0.10	0.26 ± 0.05	0.19 ± 0.07	0.060 ± 0.03
T PM	8	23.6 ± 1.3	2.5 ± 0.4 (7)	0.48 ± 0.11	0.16 ± 0.03	0.21 ± 0.05	-0.073 ± 0.05
NT PM	7	21.7 ± 1.1	4.5 ± 1.1	0.44 ± 0.06	0.23 ± 0.05	0.23 ± 0.07	0.006 ± 0.05
T LCT	8	20.3 ± 0.9	1.6 ± 0.1	0.64 ± 0.05	0.09 ± 0.01	0.07 ± 0.02	0.019 ± 0.01
NT LCT	6	19.8 ± 0.6	4.0 ± 0.9	0.64 ± 0.12	0.22 ± 0.05	0.12 ± 0.05	0.088 ± 0.03

Values are mean ± SEM. Abbreviations are as in Table III.

\*  $p < .001$  by diet (two-way ANOVA).

†  $p < .05$  T vs NT (two-way ANOVA).

‡ Statistical outlier was removed according to the Q test.

TABLE VII  
Metabolic parameters (measured during last 4 hours of parenteral feeding)

	n	Respiratory quotient	Energy expenditure (kcal/kg·day)	Plasma albumin* (g/dL)	Plasma glucose† (mg/dL)	Plasma FFA‡ (mEq/L)(n)
T SL	9	0.98 ± 0.01	186 ± 10	2.3 ± 0.0	142.9 ± 11.3	1.43 ± 0.19
NT SL	10	0.99 ± 0.01	173 ± 5	2.6 ± 0.1	143.9 ± 8.2	1.33 ± 0.18
T PM	8	0.99 ± 0.02	173 ± 12	2.8 ± 0.1	148.9 ± 12.5	0.88 ± 0.19
NT PM	8	1.01 ± 0.01	184 ± 8	2.8 ± 0.1	126.4 ± 2.2	0.41 ± 0.05
T LCT	8	1.00 ± 0.01	151 ± 9	2.7 ± 0.1	193.6 ± 33.5	1.83 ± 0.47 (7)§
NT LCT	6	1.00 ± 0.01	197 ± 15	2.8 ± 0.1	125.9 ± 8.0	0.53 ± 0.06

Values are mean ± SEM. Abbreviations are as in Table III.

\* $p < .001$  by diet (two-way ANOVA).

† $p < .05$  T vs NT (two-way ANOVA).

‡ $p < .005$  by diet (two-way ANOVA),  $p < .05$  T vs NT (two-way ANOVA).

§ Statistical outlier was removed according to the Q test.

significance of structured lipid feeding in the tumor-bearing rat is clear, these different effects of SL on the tumor and host require further investigation.

In contrast to the more positive nitrogen balance results seen in SL-fed animals, the SL group had the lowest isotope balance. Plasma albumin levels were also decreased in SL animals. Neither of these findings is consistent with previous results from our laboratory.<sup>12-14</sup> However, the percent of flux oxidized as another index of catabolism was less with the SL-fed animals. The reason for the discrepancy between these findings is not known, and additional inquiries are needed.

The present study also indicates that lipids with similar components but different structures may not have similar effects on metabolism. Although both SL and PM consist of MCTs and fish oil, SL feeding resulted in improved day 10 and cumulative nitrogen balances. On the other hand, PM daily and cumulative nitrogen balances resembled those observed in the LCT feeding group. It is uncertain why using a structured lipid vs a physical mix of MCT and fish oil would have such different effects. However, there are two possible considerations. First, SL may offer more readily available fuel for host tissue because SL contains 10% more MCT than PM (60% vs 50%), although this small difference is unlikely to have had this great an impact. Second, and more likely, uptake of the two lipids may have occurred at different rates because of their distinctly different structures. SL is made by starting with a mixture of 40% fish oil and 60% medium-chain oil by weight. The triglycerides are then hydrolyzed and reesterified, resulting in rearranged triglycerides having both medium-chain and long-chain fatty acids on the same molecule.<sup>24</sup> The reformed triglycerides consist of a mixture of the following formulations: (1) two medium chains and one long chain, (2) one medium chain and two long chains, (3) all medium chains, or (4) all long chains.<sup>25</sup> On the other hand, a physical mix is made by simply combining MCT and fish oil without any restructuring of the triglycerides, so that the molecules of PM have either all medium-chain fatty acids or all long-chain fatty acids on the triglyceride backbone. It has been reported that during intestinal absorption structured triglycerides, with medium-chain fatty acids in the 1 and 3 positions and a long-chain fatty acid in the 2 position, hydrolyze more rapidly than triglycerides composed of only long-chain

fatty acids.<sup>26</sup> If hydrolysis occurs in a similar fashion in parenterally fed animals, uptake of the diets may have occurred at different rates. The higher percentage of long-chain triglyceride molecules in PM would have slowed the intracellular uptake of this lipid. As we have previously hypothesized, SL is able to deliver abundant quantities of free fatty acids for  $\beta$ -oxidation, thereby sparing body protein.<sup>15</sup> Indeed, in the present study, the plasma free fatty acid levels were significantly higher in SL-fed groups, suggesting more rapid lipolysis by lipoprotein lipase (Table VII). In future studies this could be confirmed by measuring serum free fatty acid profiles by gas chromatography because the administered lipid has a profile vastly different from endogenous stores, and endogenous lipid mobilization would be inhibited during TPN feeding.

These metabolic pathways of SL may present the possibility that more fish oil enriched in w-3 fatty acids could be incorporated into tissues, even though SL in this study contained a lower percentage of fish oil compared with PM (40% vs 50%). We have demonstrated previously that 3 days of intravenous feeding with this SL resulted in significant increases in the w-3 fatty acid contents of all the lipid fractions in tumor-bearing rats, including amounts of 20:5w3 (eicosapentaenoic acid), 22:6w3 (docosahexaenoic acid) in phospholipids, free fatty acids, and cholesterol ester fatty acids.<sup>27</sup> Increased levels of eicosapentaenoic acid in the triglyceride fraction.<sup>23</sup> Such elevated amounts of eicosapentaenoic and docosahexaenoic acids were associated with reduced levels of arachidonic acid. If the same occurred in the present study the increased incorporation of fish oil in tumor tissues with SL feeding could alter prostaglandin production, which is one possible mechanism for improving immune functions of the host and inhibiting tumor growth.

Our study also showed that there are complex metabolic abnormalities induced by the presence of tumor in animals. First, it has been previously noted that in cancer cachexia there is insulin resistance.<sup>27</sup> In our study the significantly higher plasma glucose levels seen in tumor-bearing animals are likely due to the insulin resistance induced by tumor, although the higher free fatty acids themselves reduce glucose uptake.<sup>28</sup> Second, elevated plasma free fatty acid levels were found in tumor-bearing animals, which is in agreement with other investigators'

findings.<sup>29,30</sup> In addition, plasma leucine clearance was significantly delayed in tumor-bearing animals (Table IV). Plasma albumin levels and muscle protein fractional synthetic rates were significantly lower in tumor-bearing animals as compared with non-tumor-bearing animals, reflecting the characteristic derangements in protein metabolism seen with tumors. However, it is interesting to note that both tumor-bearing and non-tumor-bearing animals given SL TPN had similar plasma glucose and free fatty acid levels, similar leucine clearance rates, and similar muscle protein synthetic rates, suggesting that SL TPN opposes some of the effects of the tumor on intermediary metabolism.

In summary, the structuring of a triglyceride was shown to have definite biochemical benefits to host and tumor interrelationships in this experimental tumor model. If confirmed in longer term studies with more distinct slowing of tumor growth, study in humans would be warranted.

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